Synthesis of chemically-tethered amyloid- β segment trimer possessing amyloidogenic properties

Kiyomichi Shinoda^a, Youhei Sohma^{a,b,*}, and Motomu Kanai^{a,b,*}

^aGraduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

^bERATO (Japan) Science and Technology Agency (JST) Kanai Life Science Catalysis Project, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

ARTICLE INFO

Article history:

Received

Revised

Accepted

Available online

Keywords:

amyloid

oligomer

Alzheimer

aggregation

peptide synthesis

ABSTRACT

As amyloid- β (A β) undergoes dynamic aggregation, it is impossible to isolate (“hook”) the transient A β oligomer in an assembly state-pure form (*e.g.*, sole A β dimer, trimer, tetramer, etc.). Obtaining such a pure A β oligomer would allow us to establish an *in vitro* system to perform a more detailed investigation of the pathogenic properties of the oligomer. A chemically-tethered A β oligomer, constructed only by covalent bonds, could satisfy this demand. Here we designed a chemically-tethered trimer of a pathogenic A β fragment (A β_{25-35}) (**1**) and successfully generated it *in situ* from its precursor (**4**), a water-soluble and non-aggregative *O*-acyl isopeptide of **1**, in neutral aqueous media. Chemically-tethered **1** possessed stronger amyloidogenic properties, *i.e.*, potential for β -sheet structure, fibril formation, and cytotoxicity, than the corresponding monomer A β_{25-35} (**6**). Trimerization of A β_{25-35} sequence might affect both the aggregative properties and cytotoxicity, based on the present results. This work opens the door for chemical synthesis of oligomers bigger than trimers in an assembly state-pure form, allowing for identification of the most toxic A β oligomer.

2009 Elsevier Ltd. All rights reserved.

Amyloid- β peptide (A β) is widely considered the etiological cause of Alzheimer disease (AD), an incurable neurodegenerative disorder.¹ AD accounts for 60% to 70% of dementia, and there are currently more than 35 million AD patients worldwide.^{2,3} Once generated by proteolytic processing of the amyloid precursor protein, A β monomers aggregate to form oligomers and then fibrils, which eventually construct senile plaques, a pathological hallmark of AD in the brain. Among the aggregates formed by A β monomers, oligomers are the most neurotoxic,⁴ and therefore the most important potential target for the development of drugs to treat AD. The ability to further study the most toxic A β oligomer species is currently a highly attractive aim.

Despite a number of studies of mixtures of A β oligomers,⁵ none, to our knowledge, have successfully isolated an *assembly state-pure*⁶ oligomer (*e.g.*, sole A β dimer, trimer, tetramer, etc.) from a mixture of oligomers (Figure 1a). This is largely due to the extensive aggregation of A β oligomers, which results in high heterogeneity of the oligomer species. Therefore, it is technically impossible to “hook” transient oligomers composed of non-covalent bonds in an assembly state-pure form. The ability to

obtain assembly state-pure A β oligomers would allow us to set up an *in vitro* experimental system without other untargated assembly states of A β . Such a system would facilitate detailed investigations of specific oligomers and allow for the discovery of aggregation inhibitors targeting the oligomer.

One possible strategy toward achieving this aim is to synthesize chemically-tethered A β oligomers (Figure 1b). The chemically-tethered A β oligomers are constructed only by covalent bonds and not from naturally oligomer-constructing non-covalent bonds (*i.e.*, hydrogen bonds and hydrophobic interactions). Therefore, in principle, using the chemically-tethering method, we can isolate defined and unique A β oligomers in an assembly state-pure form using affordable analytical instruments. There are several previous reports of chemically-tethered A β oligomers, but they are limited to dimers^{7–11} and there has been no report on the synthesis of oligomers larger than dimers, as the synthesis of trimers is considered more difficult than that of dimers. Some reports, however, advocate an important role for A β trimers in A β pathogenicity.^{12–15} Based on this background, here we report the synthesis of A β trimers.

* Corresponding authors. Tel.: +81-3-5841-4830; fax: +81-3-5684-5206; e-mail: ysohma@mol.f.u-tokyo.ac.jp (Y. Sohma), kanai@mol.f.u-tokyo.ac.jp (M. Kanai).

Figure 1. (a) It is impossible to “hook” native A β trimers in an assembly state-pure form from a mixture of oligomers. (b) Chemically-tethered A β trimers would be obtained in an assembly state-pure form.

We planned to tether A β chains to the cyclic framework at their *N*-termini *via* flexible linkers and designed the chemically-tethered trimer (**1**; Figure 2). As the A β chain, we used the pathogenic segment A β_{25-35} , which has been used in both *in vitro*^{16–18} and *in vivo*^{19–21} experiments, and which retains the same toxic effect in rat hippocampal compared to A β_{1-42} .²²

To synthesize **1**, we prepared the following two peptides by Fmoc chemistry-based solid phase peptide synthesis: cyclic peptide **2** and azide functionalized A β_{25-35} **3** (for their structures, see Figure S1). When **2** and **3** were subjected to Cu(I)-catalyzed Huisgen cycloaddition²³ (Figure S2), the reaction mixture turned to gel as the reaction proceeded. Accordingly, the handling of **1**, which included an essential purification step by reverse-phase (RP) HPLC, was difficult, and resulted in a low isolation yield (13%). More problematic, however, was the fact that isolated **1** was not very soluble in various solvents (*e.g.*, 10% aqueous TFA, 0.02% aqueous NH₃, DMSO, hexafluoroisopropanol), and therefore we could not prepare the concentrated stock solution that was necessary for conducting the subsequent biological experiments with **1**. These observations reflected the strong aggregative properties of **1**.

Figure 2. Chemically-tethered A β_{25-35} trimer (**1**)

Therefore, we planned to apply the *O*-acyl isopeptide method²⁴ to **1**. The structure of the *O*-acyl form of **1** (**4**) is shown in Figure 3a. The *O*-acyl structure was introduced to A β chains in **1** to the bond between Gly²⁵ and Ser²⁶. The *O*-acyl form of A β_{1-42} has good solubility and no aggregative properties, whereas native A β_{1-42} has poor solubility and is strongly aggregative. Moreover, in neutral aqueous solution, *O*-acyl isoA β_{1-42} is quickly converted to native A β_{1-42} *via* an *O*-to-*N* acyl rearrangement, thus acting as a dissolvable precursor of native A β_{1-42} . We expected that **4** could act as a dissolvable precursor of **1**. Indeed, when we subjected **2** and **5** (*O*-acyl form of **3**) to the Huisgen cycloaddition, no gel was generated and **4** was obtained without difficulty *via* RP-HPLC purification with an improved isolated yield (31%, Figure S3).

Next, we prepared a stock solution of **4** to conduct physicochemical and biochemical assays investigating the aggregative properties of **1**. First, **4** was easily dissolved in 0.1% aqueous TFA affording a highly concentrated solution (150–200 μ M). To prevent any possible aggregation, this solution was subjected to ultracentrifugation (4 $^{\circ}$ C, 100,000 rpm, 1 h). The quantity of **4** was not changed by centrifugal treatment, suggesting that **4** formed no pre-aggregate species. We used this solution for several assays after diluting it with neutral (pH 7.4) aqueous media, by which condition **4** was quantitatively converted to **1** (Figure 3b), allowing us to investigate the

Figure 3. (a) *In situ* generation of **1** from **4** by an intramolecular *O*-to-*N* acyl rearrangement. (b) HPLC charts of the conversion from **4** to **1**. The HPLC analysis was performed using a C18 reverse phase column (4.6 $\times$ 150 mm; YMC-Triart C18 column) with a linear gradient of MeCN (0–100% over 40 min) in 0.1% aqueous TFA at a flow rate of 1.0 mL/min at 40 $^{\circ}$ C, and peaks were detected at 214 nm. Stock solution of **4** was diluted with 0.1% aqueous TFA and 0.1 M phosphate buffer (pH 7.4) (final concentration: 10 μ M), and the solutions were immediately subjected to analytical HPLC to obtain the charts designated as ‘before rearrangement’ and ‘after rearrangement’, respectively. The retention time of *in situ* generated **1** was identical to that of the authentic sample.

properties of **1** in the physiological environment. In size exclusion chromatography (SEC), **1** gave a single peak at the elution volume corresponding to A β ₂₅₋₃₅ trimers just after its *in situ* generation, suggesting high assembly state-purity of **1** (Figure S4).

The cross β -sheet structure is characteristic of A β aggregates, especially large oligomers and fibrils.²⁵ To compare the amount of cross β -sheet structures between **1** and the corresponding monomers A β ₂₅₋₃₅ (**6**), we performed a thioflavin-T (ThT) fluorescence assay; cross β -sheet structures are recognized by ThT, leading to an increase in its fluorescence intensity (FI) (Figure 4a).²⁶ FI in **1** (10 μ M) and in **6** (30 μ M) was identical just after incubation started ($t = 0$ h). With further incubation for up to 3 h at 37 $^{\circ}$ C, however, FI in **1** increased in a time-dependent

Figure 4. (a) ThT assay ($\lambda_{\text{ex}} = 440 \pm 5$ nm, $\lambda_{\text{em}} = 480 \pm 10$ nm). **1** (derived from **4**) or **6** was incubated at 37 $^{\circ}$ C in 0.1 M phosphate buffer (pH 7.4) for the indicated times with agitation by stirring. Mean $\pm$ s.d., $n = 3$, * $p < 0.05$ vs. **6**, ** $p < 0.01$ vs. **6** (Welch's t -test). (b) AFM images (amplitude mode). **1** (derived from **4**) or **6** was incubated at 37 $^{\circ}$ C for 20 min in 0.1 M HEPES-NaCl buffer (pH 7.4). (c) Cell viability assay. Well-seeded PC12 cells were treated with **1** (derived from **4**), **6** or **7** at 37 $^{\circ}$ C under 5% CO₂ for 4 d. Mean $\pm$ s.e.m., $n = 3$, *** $p < 0.001$ in the indicated pair (Tukey's test).

manner and almost reached a plateau at 20 min, whereas FI in **6** hardly increased at all. This finding indicated that **1** had a higher propensity to form cross β -sheet structures. As a control, chemically-tethered A β ₁₋₁₁ trimer (**9**; the sequence of A β ₁₋₁₁ is H-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-OH; for its synthesis, see Supporting Information) was synthesized. The FI in **9** was not increased (Figure S5), suggesting that the enhanced propensity of **1** to form cross β -sheet structures was not produced by the tethering. Additionally, as another control, linearly-tethered A β ₂₅₋₃₅ trimer (Ac-Lys(X)-Gly-Lys(X)-Gly-Lys(X)-Gly-NH₂ where X = triazole linker-A β ₂₅₋₃₅; **10**) was subjected to the ThT assay (for the synthesis of its *O*-acyl form (**11**), see Supporting Information). **10** showed the identical FI to **1** (Figure S6), suggesting that the contribution of the linker unit to form cross β -sheet was minor. Circular dichroism (CD) spectra also supported the predominance of β -sheet structures formed in **1**; **1** (10 μ M) changed its secondary structure from a random-coil to β -sheet-containing structure, whereas **6** (30 μ M) retained its random-coil structure (Figure S7). These findings suggest that trimerization of A β ₂₅₋₃₅ remarkably increases the propensity of cross β -sheet structure formation.

Next, to investigate whether **1** formed amyloid fibrils, we conducted atomic force microscopy (AFM). **1** (10 μ M) and **6** (30 μ M) were incubated for 20 min at 37 $^{\circ}$ C, and subjected to AFM analysis (Figure 4b; Images at $t = 10$ min and 1 h are shown in Figure S8). Under these conditions, **1** formed a larger quantity of fibrils than **6**. The morphology of the fibrils derived from **1** was robust and they were up to ca. 2 μ m long. These findings indicate that **1** had strong amyloid fibril-forming properties.

We further conducted a sedimentation assay²⁷ and SEC to investigate the aggregative properties of **1**. In the sedimentation assay, the quantity of the non-aggregated form of **1** (10 μ M) was decreased ($t = 3$ h) when incubated at pH 7.4 and 37 $^{\circ}$ C, whereas that of **6** (30 μ M) was not decreased at all (Figure S9). In SEC, both **1** and **6** gave a single peak at the corresponding elution volume at $t = 0$ h (Figure S10), but at $t = 3$ h, the peak height of **6** was almost retained, whereas the peak of **1** disappeared. The disappearance was considered to be due to the progression of aggregation to higher-molecular-weight assemblies. Such aggregates might be filtered out prior to loading onto the gel of the column. These findings were consistent with **1** having higher aggregative properties than **6**.

We considered that the stronger aggregative properties of **1** could be explained by the seeding hypothesis²⁸; conversion of monomers to oligomers is slow, but once oligomers are formed, they act to seed aggregation and the conversion of oligomers to fibrils is quite fast. The present findings are consistent with this hypothesis, as well as chemically synthesized A β dimers with stronger aggregative properties than those of the corresponding monomers.^{7,9-11}

Additionally, the activities of known aggregation inhibitors of A β were investigated to identify compounds with the potential to inhibit aggregation of **1**. Curcumin^{30,36}, EGCG^{18,29}, NQTrp (1,4-naphthoquinon-2-yl-L-tryptophan)^{31,32}, and thionin³⁴ had strong inhibitory effects on aggregation of **1**, whereas tetracycline³³ and D-H-KLVFF-OH³⁵ had no inhibitory activity (Table 1). As curcumin³⁶ and EGCG¹⁸ is known to inhibit the aggregation of A β ₂₅₋₃₅, the inhibition by EGCG implies that the aggregation pattern of the original A β ₂₅₋₃₅ is preserved in **1**. In addition, as EGCG and thionin did not exhibit such high inhibitory activities on A β ₁₋₄₂, these two compounds might have the potential to specifically inhibit aggregation of the trimers.

Table 1. Activities of aggregation inhibitors on **1** or A β_{1-42} .

Compound	ThT fluorescence intensity [%] ^a	
	1	A β_{1-42}
curcumin ^{30,36}	17 ± 8.5 ^b	5.9 ± 3.4
EGCG ^{18,29}	9.5 ± 3.3 ^c	64 ± 3.2
NQTrp ^{31,32}	2.9 ± 14	17 ± 5.4
tetracycline ³³	106 ± 14 ^d	77 ± 9.6
thionin ³⁴	0.44 ± 9.5 ^e	20 ± 0.62
D-H-KLVFF-OH ³⁵	119 ± 20	96 ± 3.1

Mean ± s.d., $n = 3-6$. ^aRelative FI to that of **1** alone (10 μ M) or A β_{1-42} alone (10 μ M), incubated in the presence of each inhibitor (50 μ M) at 37 °C for 1 h with agitation by stirring. ^b $p < 0.05$ vs A β_{1-42} (Welch's t -test). ^c $p < 0.001$ vs A β_{1-42} (Student's t -test). ^d $p < 0.05$ vs A β_{1-42} (Student's t -test). ^e $p < 0.01$ vs A β_{1-42} (Welch's t -test).

Aggregation of A β is intimately related to the onset of neurotoxicity. Accordingly, we compared the toxicity between **1** and **6** in rat pheochromocytoma PC12 cells. Each sample was added to a cell-seeded 96-well plate and the cells were incubated at 37 °C under 5% CO₂ for 4 days. Estimation of cell viability by water-soluble tetrazolium salt (WST-8) revealed significantly higher toxicity of **1** (30 μ M) compared with **6** (90 μ M) (Figure 4c). As a control, the cyclic framework **7** (30 μ M) exhibited only marginal toxicity (for synthesis of **7**, see Supporting Information). These results were highly compatible with the photographs of cells treated with **1**, **6**, and **7** (Figure S11). Thus, **1** exhibited markedly enhanced aggregation and cytotoxicity. These findings indicate that trimerization of the A β_{25-35} part of full-length A β could have pathological effects.

In summary, we developed an *in situ* generation system of the chemically-tethered A β_{25-35} trimer (**1**) using its water-soluble precursor (**4**). **1** was assembly state-pure just after its generation (SEC). **1** showed the properties of amyloidogenicity: the potential for cross β -sheet structures (ThT assay and CD), fibril formation (AFM), and cytotoxicity, and these properties were dramatically greater than that for corresponding monomer **6**. These results suggest that trimerization of A β_{25-35} sequence might affect both the aggregative properties and cytotoxicity. **1** could be potentially applied to various biological experiments as an assembly state-pure A β trimer. Moreover, we identified several aggregation inhibitors for the trimer.

This chemical-tethering method might be further applied to the synthesis of assembly state-pure A β oligomers bigger than trimers. Although an A β_{1-42} pentamer-predominant sample was previously prepared under low temperature and low salt conditions,¹⁵ other oligomers such as trimers, tetramers, etc., cannot be generated using this method. The chemical-tethering method and such condition-controlling methods might thus be used complementarily to identify the most toxic A β oligomer species. We are now working on the synthesis of chemically-tethered A β oligomers larger than trimers to obtain them in an assembly state-pure form. Moreover, if we can stabilize⁶ the chemically-tethered A β oligomers in addition to achieving their assembly state-purity, we would be able to study the toxicity of A β oligomers that do not aggregate and to assess the relationship between assembly state and toxicity. The present achievement and the forthcoming chemical synthesis-based studies of the A β oligomers will contribute to the identification of the most toxic A β oligomers.

Acknowledgments

This work was supported in part by ERATO from JST and the Takeda Science Foundation. We thank Prof. T. Katada and Dr. K. Kontani, and Prof. I. Shimada, and Dr. M. Kato at the University of Tokyo for access to the ultracentrifuge, circular dichroism spectroscopy, and atomic force microscopy and dynamic light scattering, respectively.

Supplementary Material

Supplementary data associated with this article can be found, in the online version, at doi:XXX/j.bmcl.20XX.

References and notes

- J. Hardy, D. J. Selkoe, *Science* **2002**, 297, 353–356.
- D. M. Holtzman, J. C. Morris, A. M. Goate, *Sci. Transl. Med.* **2011**, 3, 77sr1.
- H. W. Querfurth, F. M. LaFerla, *N. Engl. J. Med.* **2010**, 362, 329–344.
- D. M. Walsh, I. Klyubin, J. V. Fadeeva, W. K. Cullen, R. Anwyl, M. S. Wolfe, M. J. Rowan, D. J. Selkoe, *Nature* **2002**, 416, 535–539.
- I. Benilova, E. Karran, B. De Strooper, *Nat. Neurosci.* **2012**, 15, 349–357.
- “Assembly state-pure oligomer” in the present paper refers to the purity of the assembly state of the sample, which is given for the biological experiment. Assembly during the biological experiment is regarded as “stabilization” of the A β oligomers.
- A. Schmechel, H. Zentgraf, S. Scheuermann, G. Fritz, R. Pipkorn, J. Reed, K. Beyreuther, T. A. Bayer, G. Multhaup, *J. Biol. Chem.* **2003**, 278, 35317–35324.
- G. M. Shankar, S. Li, T. H. Mehta, A. Garcia-Munoz, N. E. Shepardson, I. Smith, F. M. Brett, M. A. Farrell, M. J. Rowan, C. A. Lemere, C. M. Regan, D. M. Walsh, B. I. Sabatini, D. J. Selkoe, *Nat. Med.* **2008**, 14, 837–842.
- W. M. Kok, D. B. Scanlon, J. A. Karas, L. A. Miles, D. J. Tew, M. W. Parker, K. J. Barnham, C. A. Hutton, *Chem. Commun.* **2009**, 6228–6230.
- T. Yamaguchi, H. Yagi, Y. Goto, K. Matsuzaki, M. Hoshino, *Biochemistry* **2010**, 49, 7100–7107.
- W. M. Kok, J. M. Cottam, G. D. Cicciostoto, L. A. Miles, J. A. Karas, D. B. Scanlon, B. R. Roberts, M. W. Parker, R. Cappai, K. J. Barnham, C. A. Hutton, *Chem. Sci.* **2013**, 4, 4449–4454.
- M. Townsend, G. M. Shankar, T. Mehta, D. M. Walsh, D. J. Selkoe, *J. Physiol.* **2006**, 572, 477–492.
- S. Matsumura, K. Shinoda, M. Yamada, S. Yokojima, M. Inoue, T. Ohnishi, T. Shimada, K. Kikuchi, D. Masui, S. Hashimoto, M. Sato, A. Ito, M. Akioka, S. Takagi, Y. Nakamura, K. Nemoto, Y. Hasegawa, H. Takamoto, H. Inoue, S. Nakamura, Y. Nabeshima, D. B. Teplow, M. Kinjo, M. Hoshi, *J. Biol. Chem.* **2011**, 286, 11555–11562.
- S. Lesné, M. T. Koh, L. Kotilinek, R. Kaye, C. G. Glabe, A. Yang, M. Gallagher, K. H. Ashe, *Nature* **2006**, 440, 352–357.
- M. Ahmed, J. Davis, D. Aucoin, T. Sato, S. Ahuja, S. Aimoto, J. I. Elliott, W. E. Van Nostrand, S. O. Smith, *Nat. Struct. Mol. Biol.* **2010**, 17, 561–567.
- B. A. Yankner, L. K. Duffy, D. A. Kirschner, *Science* **1990**, 250, 279–282.
- C. J. Pike, A. J. Walencewicz-Wasserman, J. Kosmoski, D. H. Cribbs, C. G. Glabe, C. W. Cotman, *J. Neurochem.* **1995**, 64, 253–265.
- C. Bleiholder, T. D. Do, C. Wu, N. J. Economou, S. S. Bernstein, S. K. Buratto, J. E. Shea, M. T. Bowers, *J. Am. Chem. Soc.* **2013**, 135, 16926–16937.
- J. Meunier, J. Ieni, T. Maurice, *Br. J. Pharmacol.* **2006**, 149, 998–1012.
- T. Isono, N. Yamashita, M. Obara, T. Araki, F. Nakamura, Y. Kamiya, T. Alkam, A. Nitta, T. Nabeshima, K. Mikoshiba, T. Ohshima, Y. Goshima, *Neurosci. Res.* **2013**, 77, 180–185.
- V. Lahmy, R. Long, D. Morin, V. Villard, T. Maurice, *Front. Cell. Neurosci.* **2015**, 8, 463.
- R. L. Frozza, A. P. Horn, J. B. Hoppe, F. Simão, D. Gerhardt, R. A. Comiran, C. G. Salbego, *Neurochem. Res.* **2009**, 34, 295–303.

23. V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599.
24. Y. Sohma, Y. Kiso, *Chem. Rec.* **2013**, *13*, 218–223.
25. D. A. Kirschner, C. Abraham, D. J. Selkoe, *Proc. Natl. Acad. Sci. U. S. A.* **1986**, *83*, 503–507.
26. H. LeVine III, *Methods Enzymol.* **1999**, *309*, 274–284.
27. B. O’Nuallain, A. K. Thakur, A. D. Williams, A. M. Bhattacharyya, S. Chen, G. Thiagarajan, R. Wetzol, *Methods Enzymol.* **2006**, *413*, 34–74.
28. J. D. Harper, P. T. Lansbury Jr., *Annu. Rev. Biochem.* **1997**, *66*, 385–407.
29. S. Bastianetto, Z. X. Yao, V. Papadopoulos, R. Quirion, *Eur. J. Neurosci.* **2006**, *23*, 55–64.
30. G. P. Lim, T. Chu, F. Yang, W. Beech, S. A. Frautschy, G. M. Cole, *J. Neurosci.* **2001**, *21*, 8370–8377.
31. R. Scherzer-Attali, R. Pellarin, M. Convertino, A. Frydman-Marom, N. Egoz-Matia, S. Peled, M. Levy-Sakin, D. E. Shalev, A. Caflisch, E. Gazit, D. Segal, *PLoS One* **2010**, *5*, e11101.
32. T. Zhang, W. Xu, Y. Mu, P. Derreumaux, *ACS Chem. Neurosci.* **2014**, *5*, 148–159.
33. S. Taniguchi, N. Suzuki, M. Masuda, S. Hisanaga, T. Iwatsubo, M. Goedert, M. Hasegawa, *J. Biol. Chem.* **2005**, *280*, 7614–7623.
34. G. Forloni, L. Colombo, L. Girola, F. Tagliavini, M. Salmona, *FEBS Lett.* **2001**, *487*, 404–407.
35. R. J. Chalifour, R. W. McLaughlin, L. Lavoie, C. Morissette, N. Tremblay, M. Boulé, P. Sarazin, D. Stéa, D. Lacombe, P. Tremblay, F. Gervais, *J. Biol. Chem.* **2003**, *278*, 34874–34881.
36. M. Naldi, J. Fiori, M. Pistolozzi, A. F. Drake, C. Bertucci R. Wu, K. Mlynarczyk, S. Filipek, A. De Simone, V. Andrisano, *ACS Chem. Neurosci.* **2012**, *3*, 952–962.

Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Synthesis of chemically-tethered amyloid- β segment trimer possessing amyloidogenic properties

Kiyomichi Shinoda, Youhei Sohma^{*}, and Motomu Kanai^{*}

Leave this area blank for abstract info.